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### Oral prion neuroinvasion occurs independently of PrPC expression in the gut epithelium

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1 Oral prion neuroinvasion occurs independently of PrP<sup>C</sup> expression  
2 in the gut epithelium

3

4 Running title: Prion disease in mice lacking PrP<sup>C</sup> in enterocytes

5

6 **Alison Marshall<sup>a</sup>, Barry M. Bradford<sup>a</sup>, Alan R. Clarke<sup>b,c</sup>, Jean C.  
7 **Manson<sup>d#</sup> & Neil A. Mabbott<sup>a#</sup>****

8

9 <sup>a</sup> The Roslin Institute & Royal (Dick) School of Veterinary Sciences, University of  
10 Edinburgh, Easter Bush EH25 9RG, United Kingdom

11 <sup>b</sup>, School of Biosciences, Cardiff University, Museum Avenue, Cardiff CF10 3AX,  
12 United Kingdom

13 <sup>c</sup>, Deceased

14 <sup>d</sup>, Centre for Clinical Brain Sciences, University of Edinburgh, United Kingdom

15

16 <sup>#</sup> **Address correspondence to:** Jean C. Manson, [jean.manson@roslin.ed.ac.uk](mailto:jean.manson@roslin.ed.ac.uk);

17 Neil A. Mabbott, [neil.mabbott@roslin.ed.ac.uk](mailto:neil.mabbott@roslin.ed.ac.uk)

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20 **ABSTRACT** The early replication of certain prion strains within the Peyer's patches  
21 in the small intestine is essential for the efficient spread of disease to the brain after  
22 oral exposure. Our data show that orally-acquired prions utilise specialised gut  
23 epithelial cells known as M cells to enter Peyer's patches. M cells express the  
24 cellular isoform of the prion protein, PrP<sup>C</sup>, and this may be exploited by some  
25 pathogens as an uptake receptor to enter Peyer's patches. This suggested that  
26 PrP<sup>C</sup> might also mediate the uptake and transfer of prions across the gut epithelium  
27 into Peyer's patches in order to establish infection. Furthermore, the expression  
28 level of PrP<sup>C</sup> in the gut epithelium could influence the uptake of prions from the  
29 lumen of the small intestine. To test this hypothesis, transgenic mice were created  
30 in which deficiency in PrP<sup>C</sup> was specifically restricted to epithelial cells throughout  
31 the lining of the small intestine. Our data clearly show that efficient prion  
32 neuroinvasion after oral exposure occurred independently of PrP<sup>C</sup> expression in  
33 small intestinal epithelial cells. The specific absence of PrP<sup>C</sup> in the gut epithelium  
34 did not influence the early replication of prions in the Peyer's patches or disease  
35 susceptibility. Acute mucosal inflammation can enhance PrP<sup>C</sup> expression in the  
36 intestine, implying the potential to enhance oral prion disease pathogenesis and  
37 susceptibility. However, our data suggest that the magnitude of PrP<sup>C</sup> expression in  
38 the epithelium lining the small intestine is unlikely to be an important factor which  
39 influences the risk of oral prion disease susceptibility.

40  
41 **IMPORTANCE** The accumulation of orally-acquired prions within Peyer's patches  
42 in the small intestine is essential for the efficient spread of disease to the brain.  
43 Little is known of how the prions initially establish infection within the Peyer's  
44 patches. Some gastrointestinal pathogens utilize molecules such as the cellular  
45 prion protein, PrP<sup>C</sup>, expressed on gut epithelial cells to enter Peyer's patches.

46 Acute mucosal inflammation can enhance PrP<sup>C</sup> expression in the intestine, implying  
47 the potential to enhance oral prion disease susceptibility. We used transgenic mice  
48 to determine whether the uptake of prions into Peyer's patches was dependent upon  
49 PrP<sup>C</sup> expression in the gut epithelium. We show that orally-acquired prions can  
50 establish infection in Peyer's patches independently of PrP<sup>C</sup> expression in gut  
51 epithelial cells. Our data suggest that the magnitude of PrP<sup>C</sup> expression in the  
52 epithelium lining the small intestine is unlikely to be an important factor which  
53 influences oral prion disease susceptibility.

54

55 **Key words** prions, transmissible spongiform encephalopathies, PrP, intestine, gut  
56 epithelium, Peyer's patches

57

58

## 59 Introduction

60 Prions cause chronic neurodegenerative diseases that affect humans and some  
61 domesticated and free-ranging animal species to which there are no treatments.  
62 Bovine spongiform encephalopathy (BSE) prions also have zoonotic potential (1),  
63 exerting high societal and economic costs. The precise nature of the infectious  
64 prion is uncertain, but an abnormal, relatively proteinase-resistant isoform ( $\text{PrP}^{\text{Sc}}$ ) of  
65 the host cellular prion protein ( $\text{PrP}^{\text{C}}$ ), co-purifies with prion infectivity in diseased  
66 tissues (2), and host cells must express cellular  $\text{PrP}^{\text{C}}$  to sustain prion infection (3).

67 Many natural prion diseases are acquired by oral consumption of  
68 contaminated food or pasture. The gut-associated lymphoid tissues (GALT) within  
69 the lining of the intestine such as the tonsils, Peyer's patches, appendix, colonic and  
70 caecal patches, together with the mesenteric lymph nodes (MLN), help to provide  
71 protection against intestinal pathogens. However, orally-acquired prions exploit the  
72 GALT to achieve host infection (4-8). The early replication of prions within Peyer's  
73 patches in the small intestine is essential for their efficient spread of from the gut to  
74 the brain (termed *neuroinvasion*), as oral prion disease susceptibility is blocked in  
75 their absence (5, 9-11).

76 Orally-acquired prions utilize an elegant cellular relay in the GALT in order to  
77 establish host infection. After ingestion, the prions are first transported across the  
78 follicle-associated epithelium (FAE) which covers the luminal surface of the Peyer's  
79 patches by M cells (12-16). The prions are then acquired by mononuclear  
80 phagocytes within the GALT which they appear to use as "Trojan horses" to shuttle  
81 them towards the follicular dendritic cells (FDC) in the B cell follicles (17-19). The  
82 subsequent replication of the prions upon FDC is essential for efficient  
83 neuroinvasion from the intestine (4, 5, 17, 20). The prions then infect nearby enteric  
84 nerves before spreading along fibres of the sympathetic and parasympathetic

85 nervous systems to the brain where they ultimately cause neurodegeneration and  
86 death (17, 21).

87 M cells are specialized, highly phagocytic, intestinal epithelial cells that  
88 facilitate the uptake and trans-epithelial transfer of particulate antigens and  
89 microorganisms into the GALT from the gut lumen (22). The transcytosis of  
90 particulate antigens by M cells is an important initial step in the induction of efficient  
91 mucosal immune responses against certain pathogenic bacteria (23, 24) and the  
92 commensal bacterial flora (25). However, some orally-acquired bacterial (26-28)  
93 and viral (29, 30) pathogens utilise M cells to achieve host infection. Prions also  
94 exploit M cells in order to enter Peyer's patches and establish host infection (13, 16).  
95 Furthermore, the density of M cells in the gut epithelium directly limits or enhances  
96 disease susceptibility. In the specific absence of M cells, the accumulation of prions  
97 in Peyer's patches and subsequent spread of the disease to the brain is blocked  
98 (13, 16). In contrast, increased M-cell density at the time of oral exposure enhances  
99 prion disease susceptibility approx 10 fold by increasing the uptake of prions from  
100 the gut lumen (16).

101 M cells are considered to express a variety of "immunosurveillance" receptors  
102 on their apical surfaces which enable them to acquire certain pathogens and  
103 antigens. For example, glycoprotein 2 (GP2) can act as a receptor for FimH<sup>+</sup>  
104 bacteria such as *Eschericia coli* and *Salmonella enterica* serovar Typhimurium (23).  
105 Uromodulin (also known as Tamm-Horsfall protein) may similarly mediate the  
106 uptake of surface layer protein A<sup>+</sup> lactic acid bacteria (31). Some pathogenic  
107 microorganisms appear to use receptors on M cells to aid host infection. The  
108 complement C5a receptor is expressed on the apical surface of M cells and aids the  
109 uptake of *Yersinia enterocolitica* to establish infection (32). Interactions between the  
110 type A 1 botulinum neurotoxin complex and GP2 on the M-cell surface have also

111 been shown to mediate the intestinal translocation of the toxin order to exert its toxic  
112 effects (33). M cells express the cellular isoform of the prion protein, PrP<sup>C</sup>, on their  
113 apical surfaces (26, 34). Data suggest that the pathogenic Gram-negative  
114 bacterium *Brucella abortus* utilizes the PrP<sup>C</sup> on the M-cell surface as an uptake  
115 receptor to enter Peyer's patches (26).

116 Whether the uptake and transcytosis of prions across the gut epithelium into  
117 Peyer's patches in order to establish infection predominantly occurs via constitutive  
118 sampling of the luminal contents, or via binding to specific receptors such as PrP<sup>C</sup>,  
119 is not known. Treatments that impede the early accumulation prions within the  
120 GALT can impede their spread to the brain and reduce disease susceptibility (4, 13,  
121 16, 18). Thus the identification of the molecular factors that facilitate the uptake of  
122 prions into the GALT will help the design of novel intervention targets, and enhance  
123 our understanding of the factors that influence the risk of infection. Therefore, in the  
124 current study transgenic mice were created in which *Prnp* expression (encoding  
125 PrP<sup>C</sup>) was specifically ablated in epithelial cells throughout the lining of the small  
126 intestine. These mice were then used to determine whether the absence of PrP<sup>C</sup>  
127 expression in the epithelium lining the small intestine influences oral prion disease  
128 susceptibility and the early replication of prions in the GALT.

129

130

## 131 RESULTS

132 **Conditional ablation of *Prnp* throughout the small intestinal epithelium.** The  
133 expression of Cre recombinase under the control of the rat *Cyp1a1* promoter  
134 element in *Cyp1a1*-Cre mice has been used in a series of studies to inducibly ablate  
135 the expression of *LoxP* site-flanked target genes in small intestinal progenitor cells  
136 and intestinal epithelial cells (IEC) following  $\beta$ -naphthoflavone ( $\beta$ NF) treatment (35-

37). The FANTOM5 project of the FANTOM consortium (38) has collated a large collection of cap analysis of gene expression (CAGE) data from multiple mouse tissues and cells (<http://fantom.gsc.riken.jp/zenbu>). We used this publicly available data resource to compare the expression of levels of *Cyp1a1*, *Gp2* and *Prnp* in multiple data sets derived from mouse FAE, M cells, lymphocytes and leukocytes, and brain-derived cells. This analysis confirmed that *Cyp1a1* and *Prnp* were also expressed highly in the FAE and in GP2<sup>+</sup> M cells (**Fig. 1**). However, *Cyp1a1* expression was absent in B cells, T cells and macrophages as well as brain-derived microglia, astrocytes and neurons (**Fig. 1**).

Here, *Cyp1a1*-Cre mice were crossed with *Prnp*<sup>F/F</sup> mice which carry a “floxed” *Prnp* gene (39) to enable the inducible ablation of *Prnp* specifically in IEC. Since the reliable detection of PrP<sup>C</sup> in the gut epithelium by immunohistochemistry (IHC) is technically challenging, these mice were additionally crossed with ROSA26<sup>F/F</sup> reporter mice (40) to enable the cellular-specificity of the Cre-mediated gene ablation to be readily assessed by histological assessment of  $\beta$ -galactosidase (*LacZ*) expression. The resultant progeny *Cyp1a1*-Cre ROSA26<sup>F/F</sup> *Prnp*<sup>F/F</sup> mice were termed *Prnp* <sup>$\Delta$ IEC</sup> mice, hereinafter.

Female *Prnp* <sup>$\Delta$ IEC</sup> mice were treated with  $\beta$ NF (or vehicle alone as a control) for five days to specifically ablate *Prnp* expression in IEC and tissues analyzed 14 days later. Whole-mount histological analysis showed *LacZ* expression indicative of efficient Cre-mediated gene recombination throughout the small and large intestines of  $\beta$ NF-treated *Prnp* <sup>$\Delta$ IEC</sup> mice (**Fig. 2a**). Analysis of tissue sections showed strong *LacZ* expression in IEC and crypts throughout the small intestine (**Fig. 2c**). The Cre-mediated gene recombination in the small intestinal crypts of  $\beta$ NF-treated *Prnp* <sup>$\Delta$ IEC</sup> mice was highly efficient (99.5%  $\pm$  1.1; **Fig. 2e**). In contrast, the Cre-mediated gene recombination in colonic crypts and IEC in the large intestine was



less efficient ( $64.1\% \pm 8.6$ ; **Fig. 2f**) and presented as a mosaic pattern (**Fig. 2c**). No other cellular sites of Cre-mediated recombination were observed throughout the intestines of  $\beta$ NF-treated *Prnp* <sup>$\Delta$ IEC</sup> mice. *LacZ* expression was absent within the submucosa (**Fig. 2c**), and also in the sub-epithelial dome and FDC-containing B cell-follicle regions of the GALT (**Fig. 2g**). As anticipated, no *LacZ* expression was detected throughout the small and large intestines of vehicle-treated *Prnp* <sup>$\Delta$ IEC</sup> control mice (**Fig. 2b, d, e, f, h**). *LacZ* expression was also undetectable throughout the small and large intestines of untreated *Prnp* <sup>$\Delta$ IEC</sup> control mice and  $\beta$ NF-treated *Prnp*<sup>F/F</sup> (Cre-deficient) control mice (**Fig. 2 e, f**). These data clearly demonstrate that Cre-mediated gene recombination is restricted to IEC in the small intestines of  $\beta$ NF-treated *Prnp* <sup>$\Delta$ IEC</sup> mice.

**Effect of IEC-restricted *Prnp*-ablation on prion accumulation in lymphoid tissues.** To determine the effects of IEC-specific PrP<sup>C</sup>-deficiency on oral prion disease pathogenesis, groups of female *Prnp* <sup>$\Delta$ IEC</sup> mice were treated with  $\beta$ NF for five days to specifically ablate *Prnp* expression in IEC. Untreated *Prnp* <sup>$\Delta$ IEC</sup> mice, vehicle-treated *Prnp* <sup>$\Delta$ IEC</sup> mice and  $\beta$ NF-treated *Prnp*<sup>F/F</sup> (Cre-deficient) mice were used as controls. Fourteen days later 10 mice/group were subsequently orally exposed to ME7 scrapie prions and tissues collected at 70 days post-infection. The presence of the prion disease-specific, abnormal accumulations of PrP (referred to as PrP<sup>d</sup>) which occur only in the tissues of affected animals was detected by IHC (4, 5, 11, 13, 16, 19, 41-43). However, since the IHC analysis cannot unequivocally discriminate between PrP<sup>Sc</sup> and cellular PrP<sup>C</sup>, paraffin-embedded tissue immunoblot analysis of adjacent membrane-bound sections was also used to confirm that these PrP<sup>d</sup> aggregates contained prion disease-specific, relatively proteinase-K (PK)-resistant PrP<sup>Sc</sup>. As anticipated, abundant PrP<sup>Sc</sup> accumulations were detected in

189 association with FDC (CD21/35<sup>+</sup> cells) in the Peyer's patches of control *Prnp*<sup>ΔIEC</sup>  
190 mice (**Fig. 3a**, arrows, left-hand and middle columns). Abundant FDC-associated  
191 PrP<sup>Sc</sup> accumulations were also detected in the Peyer's patches of βNF-treated  
192 *Prnp*<sup>ΔIEC</sup> mice.

193 Consistent with the IHC data (**Fig. 3a**) high levels of prion infectivity were  
194 detected in the Peyer's patches of mice from each control group (median infectivity  
195 level 6.0-6.6 Log<sub>10</sub> intracerebral [IC] infectious dose [ID]<sub>50</sub> units/g, *n* = 2-4  
196 mice/group; **Fig 3b**). IEC-restricted *Prnp*-ablation did not influence the early  
197 accumulation of infectious prions within Peyer's patches as high levels of prion  
198 infectivity were also detected in tissues from βNF-treated *Prnp*<sup>ΔIEC</sup> mice (median  
199 infectivity level 6.1 Log<sub>10</sub> IC ID<sub>50</sub> infectious units/g, *n* = 4 mice; **Fig 3b**).

200 Within weeks after oral exposure, high levels of ME7 scrapie prions first  
201 accumulate upon FDC in the Peyer's patches and are subsequently disseminated  
202 via the blood and lymph to most other lymphoid tissues including the MLN and  
203 spleen (4, 5, 11, 13, 16, 18, 19, 44). The levels of prion infectivity detected in the  
204 MLN and spleens from mice from each treatment and control group were also  
205 similar (**Fig. 3c & d**, respectively).

206 These data clearly show that IEC-restricted *Prnp*-ablation does not affect the  
207 early accumulation of orally-acquired prions within Peyer's patches or their  
208 subsequent dissemination to the MLN or spleen.

209  
210 **IEC-restricted *Prnp*-ablation does not influence oral prion disease**  
211 **susceptibility.** Female *Prnp*<sup>ΔIEC</sup> mice were treated with βNF for five days to ablate  
212 *Prnp* expression in IEC, and 14 days later subsequently orally exposed to ME7  
213 scrapie prions. Untreated *Prnp*<sup>ΔIEC</sup> mice, vehicle-treated *Prnp*<sup>ΔIEC</sup> mice and βNF-  
214 treated *Prnp*<sup>F/F</sup> (Cre-deficient) mice were used as controls. As anticipated, all of the

215 orally-exposed untreated *Prnp*<sup>ΔIEC</sup> (control) mice succumbed to clinical prion disease  
216 (mean survival time 307 ± 23 days; median 300 days, *n* = 10/10; **Table 1**).  
217 Furthermore, IEC-restricted *Prnp*-ablation did not affect disease duration (survival  
218 times) or susceptibility as all of the βNF-treated *Prnp*<sup>ΔIEC</sup> mice also succumbed to  
219 clinical prion disease with similar survival times (mean 306 ± 11 days; median 306  
220 days, *n* = 12/12, *P* = 0.673, One-way ANOVA with Dunnett's post-test; **Table 1**).

221 All the brains from the clinically-affected mice in each group displayed the  
222 characteristic spongiform pathology (vacuolation), PrP<sup>Sc</sup> accumulation, astrogliosis  
223 and microgliosis which is associated with terminal infection with ME7 scrapie prions  
224 (**Fig. 4a&b**). The severity and distribution of the spongiform pathology was also  
225 similar in the brains of the clinically-affected mice from each group (**Fig. 4c**).

226 Together these data clearly show that efficient prion neuroinvasion after oral  
227 exposure occurs independently of PrP<sup>C</sup> expression in IEC in the small intestine.

## 230 DISCUSSION

231 The initial transport of prions across the gut epithelium by M cells into small  
232 intestinal Peyer's patches is essential to establish efficient infection after oral  
233 exposure (13, 16). But whether the uptake and translocation of prions across the  
234 gut epithelium involves a specific receptor is uncertain. Treatments that prevent the  
235 initial replication of prions within the GALT impede the spread of prions to the brain  
236 and reduce disease susceptibility (4, 13, 16, 18). Thus the identification of the  
237 molecular factors that facilitate the uptake of prions into the GALT will help the  
238 design of novel intervention strategies, and enhance our understanding of the  
239 factors that influence the risk of infection. Small intestinal M cells express cellular  
240 PrP<sup>C</sup> on their apical surfaces and this may be used by certain gastrointestinal

241 pathogens as an uptake receptor to infect Peyer's patches (26, 34). Independent  
242 IHC-based tracing studies have suggested that orally-administered prion protein can  
243 be transported across the gut epithelium of PrP<sup>C</sup>-deficient mice (14, 17), but whether  
244 the expression of PrP<sup>C</sup> on IEC populations contributed to the establishment of host  
245 infection had not been assessed. Data in the current study clearly show that prion  
246 neuroinvasion after oral exposure occurs independently of PrP<sup>C</sup> expression in small  
247 intestinal IEC.

248 Orally-acquired prions replicate first in the small intestinal GALT and  
249 subsequently spread to most other secondary lymphoid tissues including the large  
250 intestinal GALT. Since oral prion disease susceptibility is substantially reduced in  
251 the specific absence of the small intestinal GALT (11), this suggests that the early  
252 replication of prions within Peyer's patches is essential to establish efficient host  
253 infection after oral exposure. The small intestinal GALT also appear to be the  
254 important early sites of prion replication in natural host species (45-47). Although  
255 we observed highly efficient Cre-mediated gene recombination in intestinal crypts  
256 and IEC throughout the small intestines of  $\beta$ NF-treated *Prnp*<sup>ΔIEC</sup> mice, the efficiency  
257 in the colon was less efficient and presented as a mosaic pattern (**Fig. 2c**) (35). The  
258 less efficient *Prnp*-ablation in the large intestine was unlikely to have influenced oral  
259 prion disease pathogenesis in the current study, as the large intestinal GALT such  
260 as the colonic patches are not important early sites of prion replication and  
261 neuroinvasion (11).

262 Despite the potentially widespread exposure of the UK population to BSE-  
263 contaminated food in the 1980s, there have fortunately there have been much fewer  
264 clinical cases of variant Creutzfeldt-Jakob disease in humans than the original  
265 estimates suggested (48) (178 definite or probable cases, as of 4<sup>th</sup> May 2018; (49)).  
266 This implies that additional factors could potentially influence an individual's

267 susceptibility to oral prion infection by enhancing or impeding the initial uptake of  
268 prions from the gut lumen. In support of this hypothesis we have shown that stimuli  
269 that increase the density of M cells in the gut epithelium also increase oral prion  
270 disease susceptibility approx 10 fold by enhancing the uptake of prions into Peyer's  
271 patches (16). The expression level of PrP<sup>C</sup> in host cells such as neurones and FDC  
272 directly influences survival times in prion infected mice (43, 50-52). Acute mucosal  
273 inflammation following oral infection with *S. Typhimurium* or treatment with dextran  
274 sodium sulphate have each been shown to enhance PrP<sup>C</sup> expression in the large  
275 intestine, implying the potential to enhance oral prion disease pathogenesis and  
276 susceptibility (53, 54). Conversely, PrP<sup>C</sup> expression was reported to be down-  
277 regulated in the small intestines of mice treated with the nonsteroidal anti-  
278 inflammatory drug indomethacin, and coincided with a modest increase in survival  
279 time after oral exposure to ME7 scrapie prions (55). Although the cellular sites of  
280 PrP<sup>C</sup> expression were not determined in the above studies, our data suggest that  
281 the magnitude of PrP<sup>C</sup> expression in IEC throughout the small intestine is unlikely to  
282 be an important factor which influences the risk of oral prion disease susceptibility.

283         In sheep with natural scrapie (56) or orally exposed to BSE prions (57), prion  
284 accumulation is first detected in the palatine tonsils in addition to the Peyer's  
285 patches. Natural prion disease susceptible host species such as sheep and cervids  
286 also have highly developed olfactory systems which they use to detect food, select  
287 mates and sense predators. A series of experimental studies in rodents and sheep have  
288 shown that prion infections can be established via the nasal cavity (58, 59) (60). Thus it  
289 cannot be excluded that soil-bound prions might also be inhaled and infect the host as the  
290 animal forages for food. Although M cells are present in the epithelia covering the nasal-  
291 associated lymphoid tissue (61), studies in hamsters indicate that these prion uptake across  
292 the nasal epithelium occurs independently of M cells (62). Whether prion uptake across

293 the mucosal surfaces in the upper gastrointestinal and upper respiratory tracts of  
294 natural host species is also PrP<sup>C</sup>-independent remains to be determined.

295 In conclusion, we show that oral prion disease neuroinvasion occurs  
296 independently of PrP<sup>C</sup> expression in IEC in the small intestine. Whether prions  
297 exploit other receptors on the apical surfaces of M cells to establish host infection is  
298 uncertain. The specific targeting of vaccine antigens to M cells has been shown to  
299 be an effective method to induce protective antigen-specific mucosal immunity (63).  
300 Mucosal immunization has also been shown to provide promising protection against  
301 oral prion infections in mice (64) and white-tailed deer (65). Thus, a thorough  
302 understanding of the mechanisms that prions exploit to establish infection within the  
303 GALT may help to identify important factors which influence the disease  
304 susceptibility, or identify novel targets for prophylactic intervention.

305

306

## 307 MATERIALS AND METHODS

308 **Mice.** The following mouse strains were used in this study where indicated:  
309 *Cyp1a1*-Cre (35); ROSA26<sup>F/F</sup> reporter strain (40); *Prnp*<sup>F/F</sup> mice (strain *Prnp*<sup>tm2Tuzi</sup>)  
310 which have *LoxP* sites flanking exon 3 of the *Prnp* gene (39). C57BL/Dk mice were  
311 also used where indicated. All mice were bred and maintained under SPF  
312 conditions. All studies and regulatory licences were approved by the Institute's  
313 ethics committee and carried out under the authority of a UK Home Office Project  
314 Licence. Prior to their use in experiments, the genotype of each mouse was  
315 confirmed by PCR analysis of tail DNA (**Table 2**).

316

317  **$\beta$ -naphthoflavone treatment.** Where indicated, mice were given five daily  
318 intraperitoneal injections of  $\beta$ -naphthoflavone (80 mg/kg; Sigma-Aldrich, Poole, UK)

319 dissolved in corn oil (Sigma-Aldrich) and analyzed 14 days after the last injection or  
320 used in subsequent experiments. Where indicated, some mice received either corn  
321 oil alone (vehicle) or no treatment as controls.

322

323 **Histological assessment of *LacZ* expression.** Tissues were first immersed in  
324 *LacZ* fixative [PBS (pH 7.4) containing 2% paraformaldehyde, 0.2% glutaraldehyde,  
325 0.02%

326 Nonidet P40, 0.01% sodium deoxycholate, 5 mM EGTA, 2 mM MgCl<sub>2</sub>] and washed  
327 in *LacZ* wash buffer [PBS (pH 7.4) containing 0.02% Nonidet P40, 0.01% sodium  
328 deoxycholate, 2 mM MgCl<sub>2</sub>]. Tissues were subsequently incubated in 15% (wt/vol)  
329 sucrose in PBS overnight followed by a further overnight incubation in 30% (wt/vol)  
330 sucrose in PBS and embedded in Tissue-Tek OCT compound (Bayer PLC,  
331 Newbury, UK). Serial sections (thickness 8 mm) were cut on cryostat and stained  
332 overnight at with *LacZ* staining solution (Glycosynth, Warrington, UK). Staining  
333 reaction was stopped by washing in *LacZ* wash buffer followed by distilled water.  
334 Sections were counterstained with nuclear fast red (Vector Laboratories,  
335 Peterborough, UK). Intestinal whole-mounts were prepared luminal side up as  
336 described previously (66), and fixed in ice-cold 2% formaldehyde/0.2%  
337 glutaraldehyde in PBS (pH 7.4) for 1 h before overnight incubation in *LacZ* staining  
338 solution.

339

340 **Prion exposure and disease monitoring.** For oral exposure, mice were fed  
341 individual food pellets dosed with 50 µl of a 1.0 % (w/v) dilution of scrapie brain  
342 homogenate (containing approximately 4.6 Log<sub>10</sub> IC ID<sub>50</sub> units) prepared from mice  
343 terminally-affected with ME7 scrapie prions according to our standard protocol (11,  
344 16, 19). During the dosing period mice were individually housed in bedding- and



345 food-free cages with water provided *ad libitum*. A single prion-dosed food pellet was  
346 then placed in the cage. The mice were returned to their original cages (with  
347 bedding and food *ad libitum*) as soon as the food pellet was observed to have been  
348 completely ingested. The use of bedding- and additional food-free cages ensured  
349 easy monitoring of consumption of the prion-contaminated food pellet. Following  
350 prion exposure, mice were coded and assessed weekly for signs of clinical disease  
351 and culled at a standard clinical endpoint. The clinical endpoint of disease was  
352 determined by rating the severity of clinical signs of prion disease exhibited by the  
353 mice. Mice were clinically scored as “unaffected”, “possibly affected” and “definitely  
354 affected” using standard criteria which typically present in mice terminal ME7  
355 scrapie prion disease. Clinical signs following infection with the ME7 scrapie prions  
356 may include: weight-loss, starry coat, hunched, jumpy behaviour (at early onset)  
357 progressing to limited movement, upright tail, wet genitals, decreased awareness,  
358 discharge from eyes/blinking eyes, ataxia of hind legs. The clinical endpoint of  
359 disease was defined in one of the following ways: i) the day on which a mouse  
360 received a second consecutive “definite” rating; ii) the day on which a mouse  
361 received a third “definite” rating within four consecutive weeks; iii) the day on which  
362 a mouse was culled in extremis. Prion diagnosis was confirmed by histopathological  
363 assessment of the magnitude of the spongiform pathology (vacuolation) in nine  
364 distinct grey-matter regions of the brain as described (67).

365 For bioassay of prion infectivity, individual tissues were prepared as 10 %  
366 (w/v) homogenates and 20 µl was injected IC into each of 4 recipient C57BL/Dk  
367 indicator mice. The prion infectivity titre in each sample was determined from the  
368 mean incubation period in the indicator mice, by reference to a dose/incubation  
369 period response curve for ME7 scrapie-infected spleen tissue serially titrated in  
370 C57BL/Dk indicator mice (68).



371

372 **Immunohistochemistry.** For the detection of disease-specific PrP (PrP<sup>d</sup>) in  
373 intestines and brains, tissues were fixed in periodate-lysine-paraformaldehyde  
374 fixative and embedded in paraffin wax. Sections (thickness, 6 µm) were  
375 deparaffinised, and pre-treated to enhance the detection of PrP<sup>d</sup> by hydrated  
376 autoclaving (15 min, 121°C, hydration) and subsequent immersion in formic acid  
377 (98%) for 5 min. Sections were then immunostained with 1B3 PrP-specific pAb. For  
378 the detection of FDC in intestines deparaffinised sections were first pre-treated with  
379 Target Retrieval Solution (DAKO) and subsequently immunostained with anti-  
380 CD21/35 (clone 7G6, BD Biosciences). Paraffin-embedded tissue immunoblot  
381 analysis was used to confirm that the PrP<sup>d</sup> detected by immunohistochemistry was  
382 proteinase K-resistant PrP<sup>Sc</sup> (69). Membranes were subsequently immunostained  
383 with 1B3 PrP-specific pAb.

384 For the detection of astrocytes, brain sections were immunostained with anti-  
385 glial fibrillary acidic protein (GFAP; DAKO, Ely, UK), and to detect microglia sections  
386 were immunostained with anti-ionized calcium-binding adaptor molecule 1 (Iba-1;  
387 Wako Chemicals GmbH, Neuss, Germany).

388 Following the addition of primary antibodies, biotin-conjugated species-  
389 specific secondary antibodies (Stratech, Soham, UK) were applied and  
390 immunolabelling was revealed using either alkaline phosphatase-conjugated to the  
391 avidin-biotin complex (Vector Laboratories, Peterborough, UK) and visualized using  
392 Vector Red (Vector Red), or HRP-conjugated to the avidin-biotin complex (Vector  
393 Laboratories) and visualized with DAB (Sigma). Sections were counterstained with  
394 haematoxylin to distinguish cell nuclei.

395

396 **Immunoblot detection of PrP<sup>Sc</sup>**

397 Brain homogenates (10% weight/volume) were prepared in NP40 lysis buffer (1%  
398 NP40, 0.5% sodium deoxycholate, 150 mM NaCl, 50 mM TrisHCL [pH 7.5]) and  
399 incubated at 37°C for 1 h with 20 µg/ml PK. Digestions were halted by addition of 1  
400 mM phenylmethylsulfonyl fluoride. Samples were then subjected to electrophoresis  
401 through 12% Tris-glycine polyacrylamide gels (Nupage, Life Technologies) and  
402 transferred to PVDF membranes by semi-dry blotting. PrP was detected using anti-  
403 mouse PrP-specific mAb 7A12 (70) followed by horseradish peroxidase-conjugated  
404 goat anti-mouse antibody (Jackson ImmunoResearch) and visualised  
405 chemiluminescence (BM Chemiluminescent substrate kit, Roche, Burgess Hill, UK).

406

407 **Statistical analyses.** Unless indicated otherwise, data are presented as mean ±  
408 SD and significant differences between groups were sought by Student's *t*-test.  
409 Values of  $P < 0.05$  were accepted as significant.

410

411

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422

423 **FIG 1** *Cyp1a1* is expressed in the follicle-associated epithelium and in M cells in the  
424 small intestine. Comparison of *Cyp1a1*, *Prnp* and *Gp2* mRNA expression in  
425 individual cell populations in deep cap analysis of gene expression (CAGE)  
426 sequence data from the FANTOM5 project of the FANTOM consortium (38). Each  
427 bar shows the relative expression level of each gene per million reads in each  
428 sample; RLE normalized tags/million). The blue hatched box highlights the small  
429 intestine-derived glycoprotein 2-expressing (GP2+) M-cell and the follicle-associated  
430 epithelium datasets. The red hatched box highlights the brain-derived datasets.

431

432 **FIG 2** Cre-mediated gene recombination is restricted to IEC in the small intestines  
433 of  $\beta$ NF-treated *Prnp* <sup>$\Delta$ IEC</sup> mice. Female *Prnp* <sup>$\Delta$ IEC</sup> mice were treated with  $\beta$ -  
434 naphthoflavone ( $\beta$ NF) for five days to specifically ablate *Prnp* expression in intestinal  
435 epithelial cells and tissues analyzed 14 days later. *Prnp* <sup>$\Delta$ IEC</sup> mice treated with  
436 vehicle alone (Veh.) were used as controls. (A, B) Whole-mount histological  
437 analysis of *LacZ* expression (blue) in the intestines of (A)  $\beta$ NF-treated *Prnp* <sup>$\Delta$ IEC</sup> mice  
438 or (B) vehicle-treated *Prnp* <sup>$\Delta$ IEC</sup> control mice. S, small intestine. L, large intestine.  
439 (C, D) Histological analysis of *LacZ* expression (blue) in IEC and crypts in the  
440 intestines of (C)  $\beta$ NF-treated *Prnp* <sup>$\Delta$ IEC</sup> mice or (D) vehicle-treated *Prnp* <sup>$\Delta$ IEC</sup> control  
441 mice. Sections were counterstained with nuclear fast red to detect cell nuclei (red).  
442 SM, submucosa. (E, F) Comparison of the % *LacZ*-expressing crypts in (E) the  
443 small and (F) large intestines, of  $\beta$ NF-treated *Prnp*<sup>F/F</sup> mice control mice. Untreated  
444 *Prnp* <sup>$\Delta$ IEC</sup> mice, vehicle-treated *Prnp* <sup>$\Delta$ IEC</sup> mice and  $\beta$ NF-treated *Prnp*<sup>F/F</sup> mice were  
445 used as controls. Data represent mean % *LacZ*-expressing crypts/mouse ( $n = 5$   
446 mice/group, 50-105 crypts/mouse). (G, H) Histological analysis of *LacZ* expression  
447 (blue) in the Peyer's patches and colonic patches of (G)  $\beta$ NF-treated *Prnp* <sup>$\Delta$ IEC</sup> mice  
448 or (H) vehicle-treated *Prnp* <sup>$\Delta$ IEC</sup> control mice.

449

450 **FIG 3** Effect of intestinal epithelial cell-restricted *Prnp*-ablation on prion  
451 accumulation in lymphoid tissues. Female *Prnp*<sup>ΔIEC</sup> mice were treated with β-  
452 naphthoflavone (βNF) for five days to specifically ablate *Prnp* expression in intestinal  
453 epithelial cells. Untreated *Prnp*<sup>ΔIEC</sup> mice and *Prnp*<sup>ΔIEC</sup> mice treated with vehicle  
454 alone (Veh.) were used as controls. Fourteen days later the mice were orally  
455 exposed to ME7 scrapie prions and Peyer's patches, mesenteric lymph nodes  
456 (MLN) and spleens collected at 70 days post infection. (A) Immunohistochemical  
457 analysis revealed high levels of disease-specific PrP (PrP<sup>d</sup>, red, middle row, arrows)  
458 were detected in association with FDC (CD21/35<sup>+</sup> cells, red, upper row) in Peyer's  
459 patches from mice from each group. Sections were counterstained with  
460 haematoxylin to detect cell nuclei (blue). Analysis of adjacent sections by paraffin-  
461 embedded tissue immunoblot analysis confirmed the presence of prion-specific PK-  
462 resistant PrP<sup>Sc</sup> (blue/black). Data representative of tissues from 6 mice/group. (B,  
463 C, D) Prion infectivity levels were assayed in (B) Peyer's patches, (C) MLN and (D)  
464 spleens from mice from each group collected at 70 days post infection. Prion  
465 infectivity titres (log<sub>10</sub> IC ID<sub>50</sub>/g tissue) were determined by injection of tissue  
466 homogenates into groups of C57BL/Dk indicator mice (*n* = 4 recipient mice/tissue).  
467 Each symbol represents data derived from an individual tissue. Red line, median  
468 prion infectivity titre for groups in which all samples contained >1 log<sub>10</sub> IC ID<sub>50</sub>/g  
469 tissue. Data below the broken horizontal line indicate disease incidence in the  
470 recipient mice <100% and considered to contain trace levels of prion infectivity.

471

472 **FIG 4.** Intestinal epithelial cell-restricted *Prnp*-ablation does not influence  
473 development of the histopathological signs prion disease in the brains of clinically  
474 affected mice. Female *Prnp*<sup>ΔIEC</sup> mice were treated with β-naphthoflavone (βNF) for

475 five days to specifically ablate *Prnp* expression in intestinal epithelial cells.  
476 Untreated *Prnp*<sup>ΔIEC</sup> mice and *Prnp*<sup>ΔIEC</sup> mice treated with vehicle alone (Veh.) were  
477 used as controls. Fourteen days later the mice were orally exposed to ME7 scrapie  
478 prions and culled when they succumbed to clinical prion disease. (A) High levels of  
479 spongiform pathology (H&E), heavy accumulations of disease-specific PrP, (PrP<sup>d</sup>,  
480 brown), reactive astrocytes expressing GFAP (brown) and active microglia  
481 expressing IBA1 (brown) were detected in the brains of all orally-exposed mice with  
482 clinical prion disease. Clin., clinical prion disease status; pos., clinically positive;  
483 individual survival times are shown (dpi, days post infection). Sections were  
484 counterstained with haematoxylin to detect cell nuclei (blue). (B) Immunoblot  
485 analysis of brain tissue homogenates confirmed the presence of high levels of prion-  
486 specific, relatively proteinase K (PK)-resistant PrP<sup>Sc</sup> within the brains of the  
487 clinically-affected mice from each group. Samples were treated in the presence (+)  
488 or absence (-) of PK before electrophoresis. After PK treatment, a typical three-band  
489 pattern was observed between molecular mass value of 20–30 kDa, representing  
490 unglycosylated, monoglycosylated, and diglycosylated isomers of PrP (in order of  
491 increasing molecular mass). (C) The severity and distribution of the spongiform  
492 pathology (vacuolation) within each brain was scored on a scale of 1–5 in nine grey  
493 matter areas: G1, dorsal medulla; G2, cerebellar cortex; G3, superior colliculus; G4,  
494 hypothalamus; G5, thalamus; G6, hippocampus; G7, septum; G8, retrosplenial and  
495 adjacent motor cortex; G9, cingulate and adjacent motor cortex. Each point  
496 represents the mean vacuolation score ± SD (*n* = 10-12 mice/group).

497

498

499

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731

**TABLE 1** *Prnp*-deficiency in the gut epithelium does not influence oral prion disease susceptibility

Mouse model <sup>a</sup>	Mean survival times (days $\pm$ SD) <sup>b,c</sup>	Median survival times (days)	Clinical disease <sup>d</sup>	Histopathological signs of prion disease in the brain <sup>e</sup>
<i>Prnp</i> <sup><math>\Delta</math>IEC</sup>	307 $\pm$ 23	300	10/10	10/10
<i>Prnp</i> <sup><math>\Delta</math>IEC</sup> + Veh.	303 $\pm$ 12	303	10/10	10/10
<i>Prnp</i> <sup><math>\Delta</math>IEC</sup> + $\beta$ NF	308 $\pm$ 11	306	12/12	12/12
<i>Prnp</i> <sup>F/F</sup> + $\beta$ NF	313 $\pm$ 19	305	9/9	9/9

<sup>a</sup> Where indicated, mice were given daily IP injections with  $\beta$ -naphthoflavone ( $\beta$ NF) or corn oil (Vehicle control, Veh.) for 5 days. Mice were orally exposed to ME7 scrapie prions 14 days after the last treatment.

<sup>b</sup> Duration from time of injection with prions to cull at clinical end-point.

<sup>c</sup> No statistical differences in survival times were observed between groups ( $P=0.673$ ; One-way ANOVA with Dunnett's post-test).

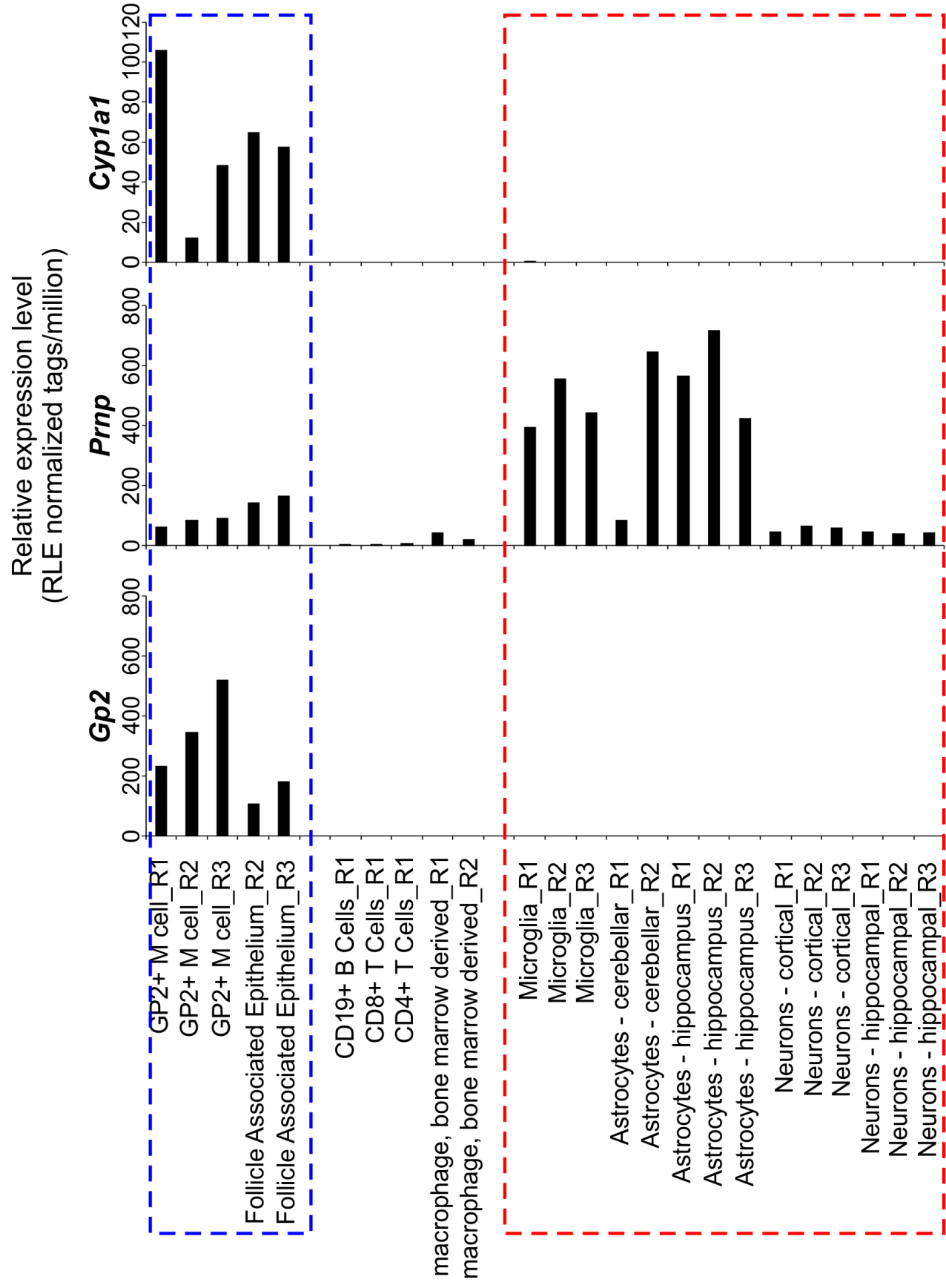
<sup>d</sup> Incidence = no. animals displaying clinical signs of prion disease/no. animals tested.

<sup>e</sup> Incidence = no. animals with histopathological signs of prion disease in the brain (spongiform pathology)/no. animals tested.

**TABLE 2** PCR primers used to confirm mouse genotypes

Allele	Details	Primer sequence	Product size/s (bp)
<i>Cre</i>	Fwd	CGAGTGATGAGGTTGCAAGAACC	786
	Rev	GCTAAGTGCCTTCTCTACACCTGC	
<i>LacZ</i>	Fwd	TACCACAGCGGATGGTTCGG	300
	Rev	GTGGTGGTTATGCCGATCGC	
<i>Prnp<sup>flox</sup></i>	1	AATGGTTAACTTTCGTTAAGGAT	Recombined <i>Prnp<sup>F</sup></i> 344
	2	GCCGACATCAGTCCACATAG	<i>Prnp<sup>F</sup></i> 210
	3	GGTTGACGCCATGACTTTC	<i>Prnp<sup>+</sup></i> 167
<i>Prnp<sup>+</sup></i>	Fwd	TCATCCCACGATCAGGAAGATGAG	600
	Rev	ATGGCGAACCTTGGCTACTGGCTG	

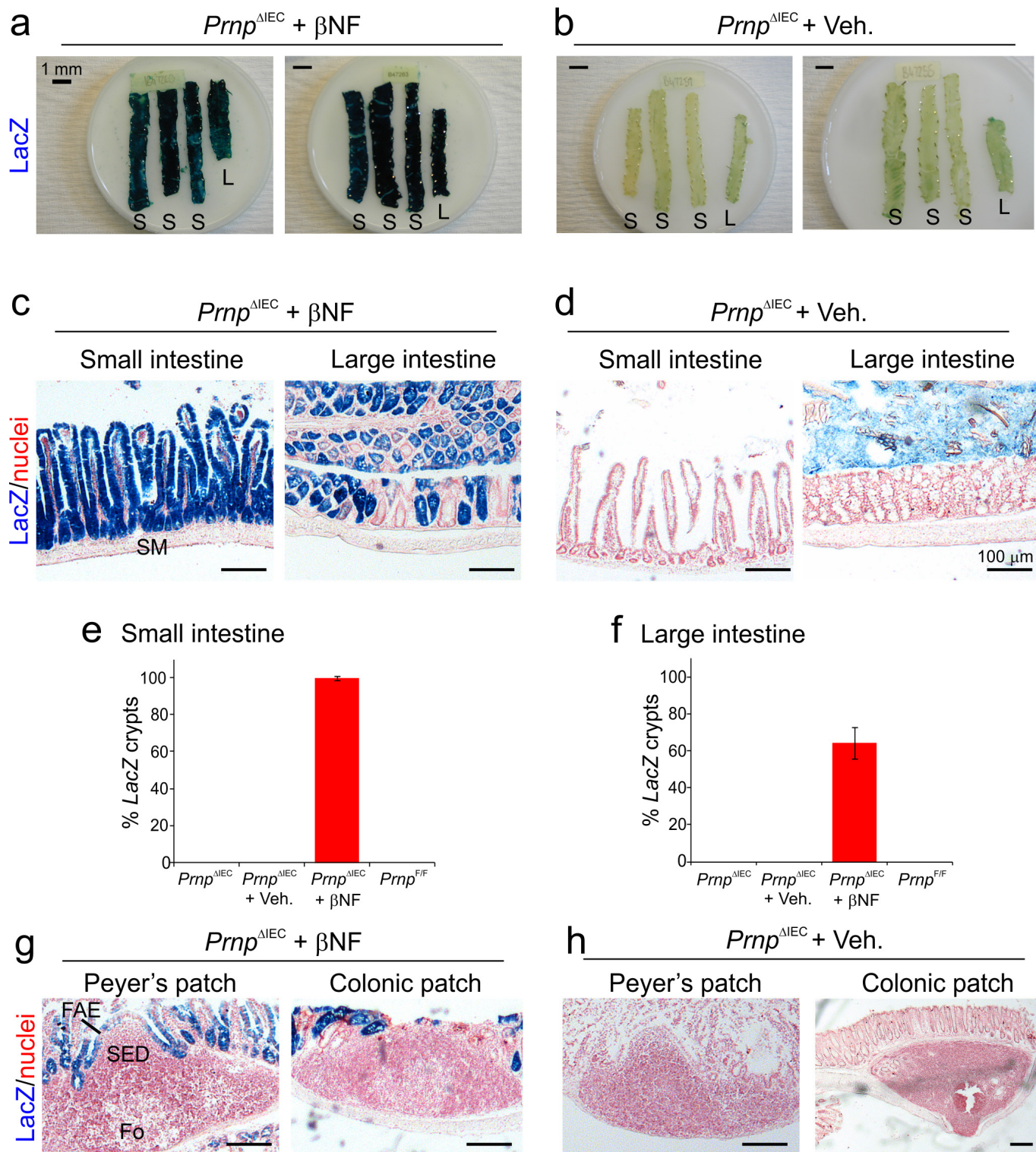
Fwd, forward primer; Rev, reverse primer; Recombined *Prnp<sup>F</sup>*, Cre-mediated DNA recombined allele



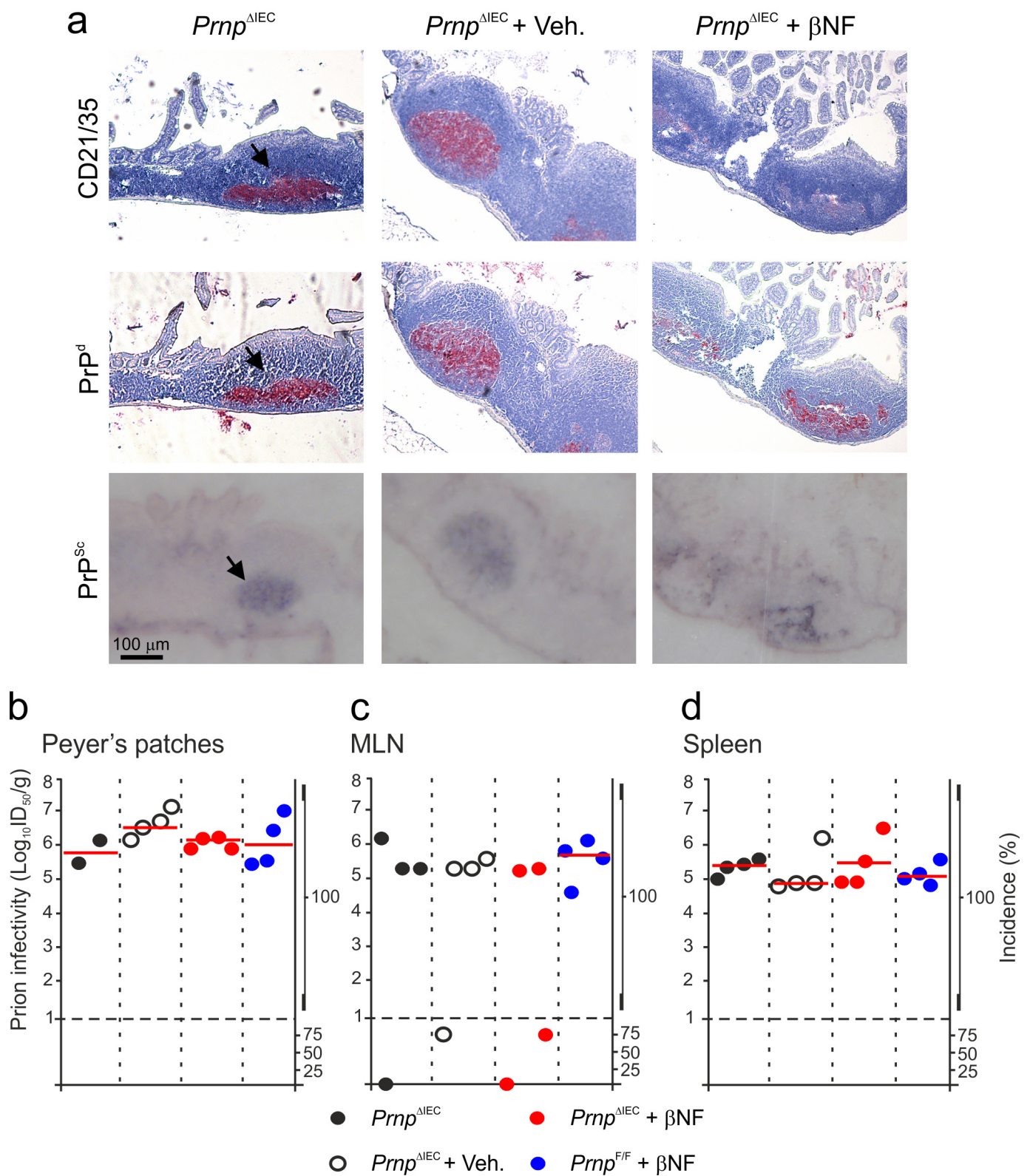
Marshall Fig.1



Marshall Fig.2



Marshall Fig.3





Marshall Fig. 4

